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Time-Resolved Immunofluorometric Assay for the Quantification of Lipoprotein(a) in Serum

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Summary: Although two recent studies have failed to reveal lipoprotein(a) (LP(a)) serum concentrations > 300 mg/l to be an independent risk factor for early onset of atherosclerosis, Lp(a) serum concentrations are frequently measured to evaluate the additional risk of coronary heart disease. We describe a time-resolved immunofluorometric assay (TRIFMA) for quantifying Lp(a) levels in human serum using commercially available reagents, which is rapid, robust and simple to perform. The two-site immunometric assay was based on microtitre plates as solid phase coated with a polycloncal anti Lp(a) antibody. The liquid-phase antibody was labelled with biotin and detected by europium labelled streptavidin in the DELFIA 1232 fluorometer. The measuring range was 2–1600 mg/l. The intra-assay imprecision was < 7% (CV), the inter-assay imprecision < 12% (CV). No interference was detected with plasminogen concentrations up to 2.2 g/l. There was an acceptable correlation with a commercially available enzyme immunoassay (r = 0.95) and with electroimmunodiffusion (r = 0.85) on 100 routine serum samples measured. The assay appeared to detect different Lp(a) isoforms as dilution curves were parallel for B/F, S_2 and S_4 isoforms.

Introduction

Numerous epidemiological and case-control studies have revealed lipoprotein(a) (Lp(a)) serum concentrations > 300 mg/l to be an independent risk factor for myocardial infarction, stroke and restenosis after coronary artery bypass surgery (1–9). The results have been confirmed by a prospective study (10). *Ridker* et al. and *Jauhiainen* et al., however, could not show any correlation between Lp(a) serum levels and atherosclerosis (11, 12). Nevertheless, Lp(a) serum concentrations are frequently measured to estimate the additional risk of atherosclerotic disease.

Lp(a)[1]) is a cholesterol ester-rich lipoprotein which resembles LDL[1]) with the presence of apolipoprotein B-100 to which the glycoprotein apolipoprotein(a) is covalently bound. Apolipoprotein(a) contains three structural regions, which are also present in plasminogen: variable numbers of kringle 4, one kringle 5 and a protease domain (14). The number of kringle 4 repeats is a genetic trait and determines the relative molecular mass (M_r) polymorphism of apolipoprotein(a). In the Caucasian population the frequency distribution of Lp(a) serum concentration is markedly skewed with a shift toward lower levels. Lp(a) serum concentrations are inversely related to the size of apolipoprotein(a) isoforms and range from less than 1 mg/l to > 2000 mg/l (9).

Several analytical methods have been described to quantify Lp(a) levels in serum. Electroimmunodiffusion (EID) was followed by radioimmunoassay (RIA) and enzyme immunoassay (EIA) (16–20). Nephelometric techniques have recently been introduced to automate the Lp(a) determination (21, 22). These methods, however, are either difficult to perform in large series, time consuming, or lack accuracy and precision at low and

[1]) Non-standard abbreviations:
Lp(a): lipoprotein(a); TRIFMA: time-resolved immunofluorometric assay; LDL: low density lipoprotein; IRMA: immunoradiometric assay; EIA: enzyme immunoassay; IEMA: immunoenzymometric assay.

high concentrations of Lp(a). The use of radioimmunoassays is restricted to authorised laboratories.

We describe a non-radioisotopic Lp(a) assay, which offers a wide measuring range, high sensitivity and low intra- and inter-assay imprecision. The assay is rapid, robust, simple to perform and suitable for routine use.

Materials and Methods

Samples

Serum was used as specimen throughout. The sera were measured either directly after centrifugation or after being stored frozen at −20 °C. Samples to define the reference range were obtained from healthy blood donors. The plasma and serum samples for the comparison of measurement in plasma and serum were obtained from healthy employees of the hospital.

Reagents

All chemicals were analytical grade from Merck (Darmstadt, Germany) or Sigma (Deisenhofen, Germany), if not stated otherwise. We used double distilled water for all buffers and solutions.

Buffers

Assay buffer

Tris 0.05 mol/l, NaCl 0.15 mol/l, bovine serum albumin 5 g/l (Sigma), bovine γ-globulin 0.5 g/l (Sigma), diethylenetriaminepentaacetic acid (DTPA) 0.01 mol/l (Sigma), NaN_3 0.15 mmol/l, Tween 20 0.2 ml/l (Sigma), pH adjusted to 7.75.

Washing buffer

Tris 0.05 mol/l, NaCl 0.15 mol/l, Tween 20 1 ml/l, pH 7.5. The washing buffer was stored in a 40-fold concentrate and was stable for half a year.

Coating buffer

Na_2CO_3 0.05 mol/l, $NaHCO_3$ 0.05 mol/l, NaN_3 0.15 mmol/l, pH 9.6.

Enhancement solution

Citric acid 0.1 mol/l, tri-n-octyl-phosphine-oxide 0.01 mol/l, potassium-phthalate 0.08 mol/l (Sigma), thenoyltrifluoroacetone 0.01 mol/l (Sigma), Triton X-100 2 ml/l (Sigma).

Antibodies

The polyclonal Lp(a) antibodies were purchased from DAKO (Hamburg, Germany), Code No. Q 023, Lot No. 062, and from Immuno GmbH (Heidelberg, Germany) Art. No. 4845009, Lot No. 2421/360. The calibrator and the controls were from Immuno GmbH, Art. No. 4395006 and Art No. 4395106, respectively.

Streptavidin was purchased from Biomol (Hamburg, Germany), the europium labelling reagent was from Pharmacia (Uppsala, Sweden), Art. No. 1244-301. Microtitre plates were from Nunc (Roskilde, Denmark), maxisorp F 96.

Apparatus

We used the Novopath Platewasher (Bio Rad, Munich, Germany), the Heidolph microtitre plate shaker (Heidolph, Kelheim, Germany), and the DELFIA 1232 time-resolved fluorometer from Pharmacia (Uppsala, Sweden).

Assay procedure

Microtitre plates were coated with 200 µl per well of sheep anti Lp(a) antibody from Immuno (10 µl diluted in 20 ml coating buffer). Each plate was allowed to stand overnight at 4 °C, then the coating solution was aspirated, the plate washed once with washing buffer, and 200 µl per well of assay buffer were added for storage.

Calibrators, controls and samples were diluted 1 : 500 in assay buffer. The calibrator containing 800 mg/l Lp(a) was diluted 1 : 250 to give a final concentration of 1600 mg/l. Twenty µl of the diluted calibrators, controls and samples were pipetted into each well, allowed to stand for 30 minutes at ambient temperature (18−22 °C) followed by 200 µl assay buffer. After incubation for one hour on the microtitre plate shaker with 200 min^{-1} at ambient temperature, the plate was washed three times with washing buffer.

Tab. 1 Influence of haemolysis on the Lp(a) concentrations measured with the Lp(a) TRIFMA. Ten serum samples were measured directly and after mechanical treatment to produce haemolysis. On the right the haemoglobin concentration in the sera after haemolysis is given. The coefficient of correaltion for the Lp(a) concentrations before and after haemolysis was r = 0.998.

Sample no.	Lp(a) concentration		Haemoglobin concentration
	before haemolysis [mg/l]	after haemolysis [mg/l]	[g/l]
1	60	57	1.2
2	420	390	1.4
3	620	600	1.8
4	260	270	1.9
5	9.9	9.0	1.6
6	440	450	1.2
7	590	605	1.1
8	16	14	1.4
9	245	238	1.3
10	58	55	1.9

Tab. 2 Influence of triacylglycerol concentrations on the Lp(a) concentrations measured with the Lp(a) TRIFMA. One sample containing high level of Lp(a) (496 mg/l) and low level of triacylglycerol (0.3 mmol/l) was mixed with another sample with low Lp(a) concentration (47 mg/l) and high level of triacylglycerol (15.7 mmol/l). No influence of triacylglycerol concentration on the Lp(a) concentrations can be observed.

Fraction of		Lp(a) concentration [mg/l]	
sample 1	sample 2	Measured	Expected
1.00	0	496	496
0.90	0.10	447	451
0.80	0.20	387	406
0.70	0.30	344	361
0.60	0.40	294	316
0.50	0.50	246	272
0.40	0.60	203	227
0.30	0.70	168	182
0.20	0.80	122	137
0.10	0.90	86	92
0	1.00	47	47

Two-hundred µl of DAKO anti Lp(a) antibody which had been labelled with biotin as described previously (26) and diluted in assay buffer to a final concentration of 1 mg/l were incubated for another hour (ambient temperature, 200 min^{-1}). After washing three times with washing buffer, 200 µl of the streptavidin europium conjugate in assay buffer were added to each well and incubated for 30 minutes (ambient temperature, 200 min^{-1}). Streptavidin was labelled as recommended by Pharmacia (Uppsala, Sweden) using their europium labelling reagent and diluted to a final concentration of 0.025 mg/l. After a final washing step 200 µl enhancement solution were added to each well and then incubated for 30 minutes (ambient temperature, 200 min^{-1}). The plate was measured in the DELFIA 1232 fluorometer. The calibration curve was constructed using a modified spline function in the FIA-Calc data reduction programme of the DELFIA 1232 system after a log/log transformation of the data.

Tab. 3 Lp(a) levels in serum, heparin-, citrate- and EDTA-plasma of 20 healthy hospital employees. The coefficients of correlation for serum vs. citrate-plasma was r = 0.994, for serum vs. heparinized plasma r = 0.998, for serum vs. EDTA-plasma r = 0.999, respectively. (* Dilution effect of 1 : 10 must be taken into consideration. These were the measured concentrations.)

Sample no.	Lp(a) concentration [mg/l] Serum	Lp(a) concentration [mg/l]* Citrate-plasma	Lp(a) concentration [mg/l] Heparin-plasma	Lp(a) concentration [mg/l] EDTA-plasma
1	54	46	50	51
2	143	124	94	134
3	22	20	21	15
4	71	60	60	63
5	100	78	96	111
6	33	27	34	30
7	25	23	28	26
8	19	17	15	17
9	12	14	14	13
10	360	260	300	330
11	130	110	100	110
12	22	20	22	23
13	9	9	8	8
14	59	46	52	58
15	1400	1560	1200	1240
16	267	257	296	285
17	31	27	29	33
18	320	230	290	290
19	16	13	13	19
20	43	33	38	34

Tab. 4 Influence of storing conditions of Lp(a) measurement. Ten serum samples stored at 4 °C and frozen at −20 °C for 21 days, respectively. Another ten samples have been stored at 4 °C and at −80 °C for the same time. The coefficients of correlation for Lp(a) concentrations measured in samples stored at 4 °C vs. samples stored at −20 °C was r = 0.996, for samples stored at 4 °C vs. −80 °C r = 0.990, respectively.

Sample no.	Storing at 4 °C Lp(a) [mg/l]	Storing at −20 °C Lp(a) [mg/l]	Sample no.	Storing at 4 °C Lp(a) [mg/l]	Storing at −80 °C Lp(a) [mg/l]
1	32	26	11	379	417
2	110	130	12	197	201
3	451	426	13	490	570
4	40	33	14	34	31
5	>1600	>1600	15	116	139
6	14	14	16	83	93
7	747	671	17	40	39
8	984	836	18	155	163
9	24	21	19	35	32
10	387	358	20	257	216

Influence of haemolysis

To investigate the influence of haemolysis 10 blood samples were divided into two aliquots each. One was immediately centrifuged and the serum measured. The other was mechanically haemolysed on a tube shaker. All samples were macroscopically haemolytic after this procedure. The haemoglobin concentration after centrifugation was between 1 and 2 g/l, as measured with the cyanmethaemoglobin method (tab. 1).

Tab. 5 Stability of the coated Lp(a) microtitre plate stored for 3 months in assay buffer at 4 °C. Given are the calibrator concentrations, the response and the concentrations of 3 control sera measured with plates prepared 1 week and 13 weeks before. (* Assay date for both plates was 7. Feb. 1995.)

Calibrator concentration [mg/l]	Response [counts/s]* Plate coated 28. Oct. 1994	Plate coated 6. Feb. 1995
2	9071	9440
10	30414	32016
50	118040	122472
200	365959	388782
800	877622	968806
1600	1207095	1209315
	Concentration [mg/l]	
Control 1	187	190
Control 2	378	345
Control 3	683	715

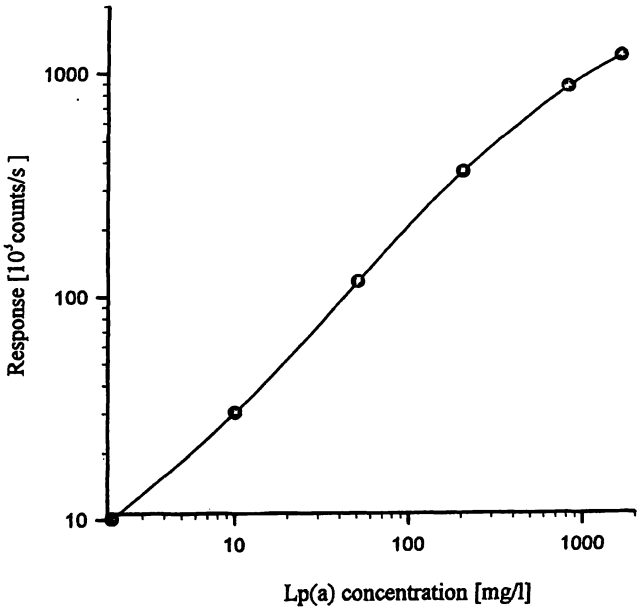

Fig. 1 Calibration curve of the Lp(a) TRIFMA after log/log transformation of the data.

Tab. 6 Analytical recovery of the Lp(a) TRIFMA. Eighteen serum samples were mixed 1 + 1 (by vol.) with a serum containing 950 mg/l Lp(a). The analytical recovery was calculated in percent.

Sample no.	Serum Lp(a) concentration [mg/l]	Added concentration [mg/l]	Expected value [mg/l]	Measured value [mg/l]	Recovery [%]
1	403	950	675	675	100
2	201	950	575	598	102
3	100	950	525	501	95
4	7	950	478	429	87
5	640	950	507	503	99
6	995	950	973	1016	104
7	7	950	478	427	89
8	108	950	500	542	108
9	543	950	745	378	99
10	241	950	595	564	95
11	10	950	480	453	94
12	77	950	510	486	95
13	52	950	501	457	91
14	8	950	479	479	100
15	298	950	624	613	98
16	389	950	669	633	95
17	204	590	575	606	106
18	19	950	480	448	93

Influence of lipaemia

To investigate the influence of hypertriacylglycerolaemia 2 samples were mixed with each other in steps of 10%. Sample 1 contained 496 mg/l Lp(a) and 0.3 mmol/l triacylglycerols as determined in the routine laboratory. Sample 2 contained 47 mg/l Lp(a) and 15.7 mmol/l triacylglycerols, respectively (tab. 2).

Interference of plasminogen

To assess the interference of plasminogen on the assay purified plasminogen (Sigma) was diluted in assay buffer to give final concentrations of 0.1; 2.2; 22; 220 and 2200 mg/l, respectively and measured in the assay. All concentrations were below the lowest calibrator (0.06; 0.26; 1.1; 0.6 and 1.4 mg/l, respectively). Even when measured without the obligate predilution corresponding to a theoretical, original concentration of 1100 g/l(!) only 16 mg/l were detected.

Measurement in serum and different kinds of plasma

To evaluate the effect of measuring Lp(a) in different derivatives from blood, blood from 20 healthy employees of the hospital was obtained as serum, heparinized plasma, EDTA-plasma and citrate-plasma. Results are given in table 3.

Effect of storing conditions on Lp(a) levels

To assess the influence of storing conditions on the stability of Lp(a) 10 serum samples were stored at 4 °C and −20 °C for 21 days. Another 10 samples were stored at 4 °C and −80 °C for the same period (tab. 4). The effect of repeated freeze and thaw cycles was not investigated, because the negative influence is well known.

Stability of coated microtitre plates

To evaluate the stability of the coated microtitre plates one plate was stored for 13 weeks in assay buffer at 4 °C and compared with a plate coated one week before (tab. 5).

Electroimmunodiffusion (EID)

Electroimmunodiffusion was performed in a 1 mm, anti Lp(a) antiserum containing agarose gel with a LKB-Multiphor II electrophoresis unit (LKB-Pharmacia, Uppsala, Sweden) as described recently (16, 23).

Enzyme immunoassay (EIA)

To quantify Lp(a) by EIA, the IMMUNOZYM Lp(a) EIA kit from Immuno GmbH (Heidelberg, Germany) was used following the manufacturers recommendations. This kit uses a monospecific, polyclonal anti apolipoprotein(a) antibody on the solid-phase, and a peroxidase labelled, monoclonal anti apolipoprotein(a) antibody for detection (figs. 3 and 4).

Influence of apolipoprotein(a) isoforms on Lp(a) measurement

To investigate, whether the TRIFMA measures irrespective of different isoforms of apolipoprotein(a), sera containing apolipoprotein(a) isoforms B/F, S_2 and S_4 (determined as described by *Laemmli* (29)) were measured after serial dilution in assay buffer (Fig. 5).

Measurement in triacylglycerol-rich fraction

To test Lp(a) measurement in triacylglycerol-rich fraction of serum, sera were ultracentrifuged at 30 000 min^{-1} for 18 hours and

Tab. 7 Intra-assay and inter-assay imprecision of the Lp(a) TRIFMA.

Mean concentration [mg/l]	Intra-assay imprecision CV [%]	Mean concentration [mg/l]	Inter-assay imprecision CV [%]
61 (n = 20)	2.81	49 (n = 22)	11.5
372 (n = 20)	4.10	193 (n = 22)	6.64
932 (n = 20)	6.77	776 (n = 22)	9.83

the supernatant and the remaining serum fraction measured separately after serial dilution in assay buffer (fig. 6).

Statistical analyses

The correlation of Lp(a) levels between TRIFMA, EID and EIA, was calculated using a non-parametric linear regression analysis as described by *Passing & Bablock* (30). In the legends to the figures 2, 3 and 4 the coefficient of correlation (r), the equation formula of the regression analysis (y = a + bx), the result of the Cusum test for deviation from linearity (significant or not significant) and the result of the test for contingency (p-values) are given. To prove the data in tables 1, 3 and 4 statistically, the coefficient of correlation was calculated, instead of a statistical test, because of the low number of data pairs.

Results

Assay characteristics

Figure 1 shows a typical calibration curve for the Lp(a) TRIFMA. The potential lower detection limit was 0.39 mg/l, calculated as the response of the zero calibrator (assay buffer) + 3 SD (n = 20). The measuring range was 2−1600 mg/l. A high dose "hook" effect was not detectable up to 5000 mg/l (response for the 1600 mg/l calibrator: 1 014 527 counts/s, response for 5000 mg/l: 1 689 754 counts/s). Analytical recovery ranged from 87 to 108% (tab. 6). The intra-assay imprecision was < 7% (CV, n = 20), the inter-assay imprecision was < 12% (CV, n = 22) (tab. 7).

To calculate the reference range of Lp(a) in this assay, specimens from 88 healthy blood donors were measured. There is a wide range of Lp(a) levels in healthy individuals which does not follow a *Gaussian* distribution, as previously described (13). Thus, we calculated the median, the 75th, 90th and 95th percentile instead of the arithmetic mean. The median of the 88 samples was 99 mg/l, which corresponds well with the literature (24), the 75th percentile was 362 mg/l, the 90th 569 mg/l and the 95th 670 mg/l Lp(a), respectively. There was no interference with plasminogen detectable up to a plasminogen concentration of 2.2 g/l (10 fold above the upper limit of the reference range) and no interference with lipaemic or haemolytic samples, either (tabs. 1 and 2).

The Lp(a) TRIFMA was compared with the EIA from Immuno and the electroimmunodiffusion technique. The results of the TRIFMA correlated highly with the EIA (r = 0.95; y = 1.47 x + 7.66, Cusum test on deviation from linearity: not significant, test for contingency: p = 0.003, n = 100; fig. 2). Differences were seen in low (< 40 mg/l) and high concentrations (> 450 mg/l). The correlation between the results of the TRIFMA and the electroimmunodiffusion assay (EID) was not as good as the latter, probably because of the better linearity of the TRIFMA over a wide measuring range (r = 0.85; y = 1.38 x − 69.7; Cusum test on deviation from linearity: not significant, test for contingency: p = 0.308, n = 100; fig. 3). This conjecture is supported by the better correlation between EIA and EID (r = 0.92; y = 0.98 x − 61.6; Cusum test on deviation from linearity: not significant, test for contingency: p = 0.065, n = 100; fig. 4).

Figure 5 shows the influence of different apolipoprotein(a) isoforms on the Lp(a) measurement with the Lp(a) TRIFMA. The distribution of Lp(a) between the

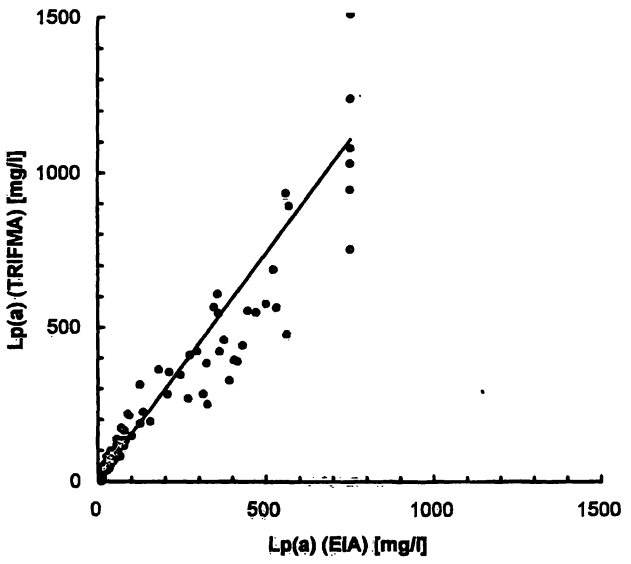

Fig. 2 Plot of the regression analysis of Lp(a) serum levels as determined by enzyme immunoassay (EIA = x) and time-resolved immunofluorometric assay (TRIFMA = y) (r = 0.95; y = 1.47 x + 766; as described by *Passing & Bablok*, Cusum test on deviation from linearity: not significant, test for contingency: p = 0.003, n = 100).

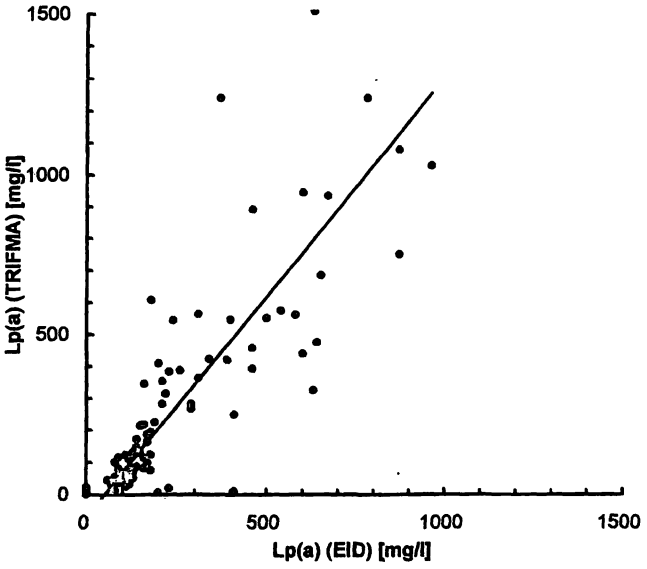

Fig. 3 Plot of the regression analysis of Lp(a) serum levels [mg/l] as determined by electroimmunodiffusion (EID = x) and time-resolved immunofluorometric assay (TRIFMA = y) (r = 0.85; y = 1.28 x − 69.7; as described by *Passing & Bablok*, Cusum test on deviation from linearity: not significant, test for contingency: p = 0.308, n = 100).

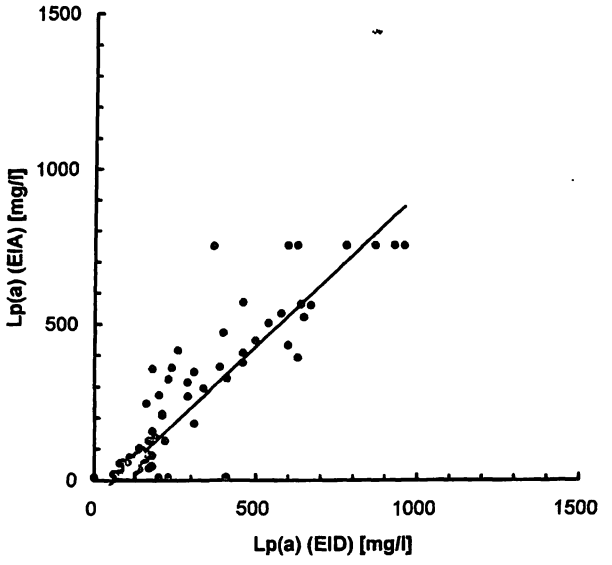

Fig. 4 Plot of the regression analysis of Lp(a) serum levels as measured by electroimmunodiffusion (EID = x) and enzyme immunoassay (EIA = y) (r = 0.92; y = 0.98 x − 61.6; as described by *Passing & Bablok*, Cusum test on deviation from linearity: not significant, test for contingency: p = 0.065, n = 100).

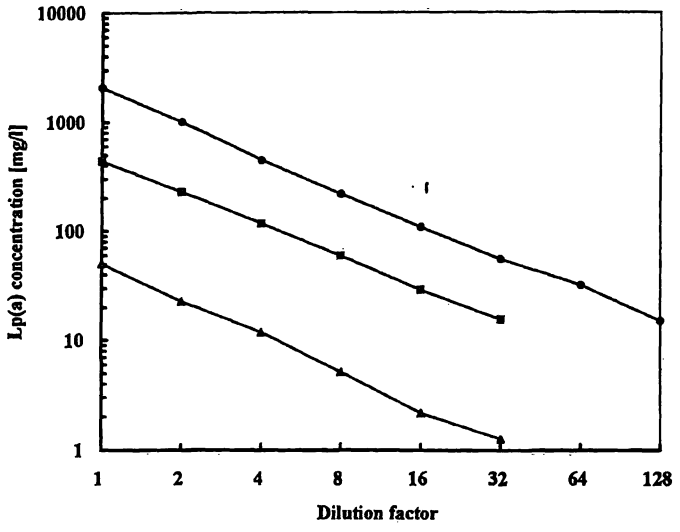

Fig. 5 Influence of different apolipoprotein(a) isoforms on the Lp(a) measurement with the Lp(a) TRIFMA. Three sera were serially diluted in assay buffer and Lp(a) concentrations were measured. Serum 1 (●) had a Lp(a) concentration of 2000 mg/l, the apolipoprotein(a) isoform was determined as B/F, serum 2 (■) had a Lp(a) concentration of 440 mg/l, apolipoprotein(a) isoform was S_2, serum 3 (▲) contained 50 mg/l Lp(a) and apolipoprotein(a) isoform S_4.

supernatant and the remaining fluid after ultracentrifugation is shown in figure 6.

Discussion

Numerous investigations have revealed Lp(a) to be a genetically determined, independent risk factor for coronary heart disease, cerebrovascular disease and peripheral arterial occlusion (1−9) although two studies, however, have failed to confirm these results (11, 12). Lp(a) serum levels are quantified routinely with different analytical methods.

Electroimmunodiffusion (EID) is often referred to as the "standard" method to measure Lp(a) (15, 16) and has been used to investigate Lp(a) in many studies. It is a simple and robust method, which is on the one hand not sensitive enough to detect low levels of Lp(a) and on the other hand not suitable for measuring large numbers of samples. Only 25 samples can be applied per gel and it takes two days until results are available. The nephelometric and turbidimetric assays can be completely automated using modern nephelometers but difficulties can arise with lipaemic or haemolytic sera (13). Furthermore, Lp(a) with its inverse relation between particle size and serum concentration is not the most suitable analyte for these techniques. These problems do not occur in sandwich immunoassays using either enzyme linked or radiolabelled antibodies for detection. Immunoradiometric assays (IRMA) and immunoenzymometric assays (IEMA) show excellent sensitivity and specificity (16−20). They are capable of assaying large numbers of samples. The use of radiolabelled substances, however, limits the suitability of such methods for routine laboratory application.

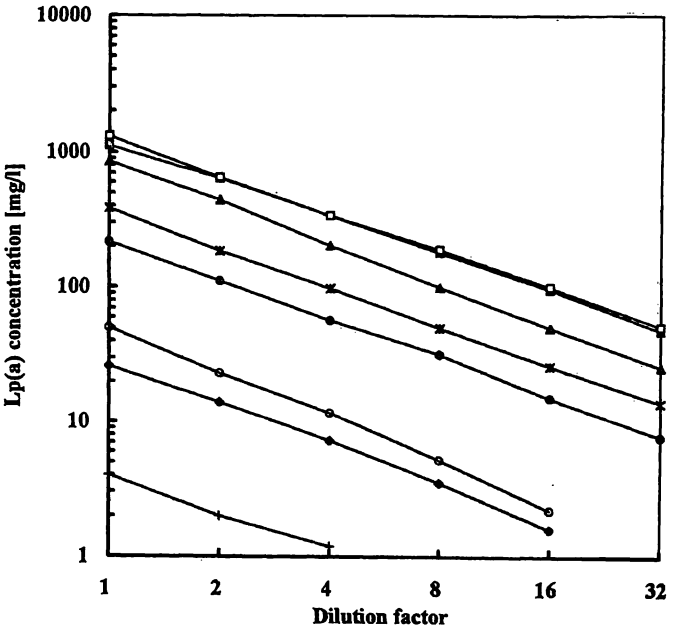

Fig. 6 Four sera were ultracentrifuged at 30 000 min^{-1} for 18 hours. The supernatant containing the triacylglycerol-rich fraction was separated and the supernatant and the remaining serum were separately measured in the Lp(a) TRIFMA after serial dilution in assay buffer. Serum I contained 26 mg/l Lp(a) in the supernatant (◆) and 4 mg/l in the remaining fluid (+), serum II contained 848 mg/l (▲) and 384 mg/l (✳), serum III 1110 mg/l (■) and 1307 mg/l (□) and serum IV 216 mg/l (●) and 50 mg/l (○), respectively.

Our data show, that Lp(a) has been stable for at least 3 weeks at 4 °C, −20 °C and −80 °C (tab. 4). The calibrators and controls have been stored for 2 years at −80 °C without any loss of immunoreactivity. This is in agreement with the experience of other investigators ((23) and *Wood*, personal communication). Therefore, we cannot support the experience of *Craig* et al. (27) as far as two-site immunometric assays are concerned. The effect of freezing and thawing has been described previously (28).

The different immunoreactivity of the Lp(a) isoforms remains a problem in Lp(a) quantification. In our studies dilution experiments with samples containing different apolipoprotein(a) isoforms showed linear dilution curves irrespective of the apolipoprotein(a) isoform, when measured with our method. Thus, it can be concluded, that the TRIFMA measures Lp(a) independently of apolipoprotein(a) isoforms. This may be explained by the use of two polyclonal antibodies in this assay. Furthermore, dilution experiments after separating the triacylglycerol-rich fraction of serum and measuring both fractions separately showed linear and parallel dilution curves in different Lp(a) concentration ranges irrespective of the serum fraction (fig. 6). These data suggest, that the TRIFMA can also measure free apolipoprotein(a). This can, however not be proved exactly due to lack in availability of free apolipoprotein(a), although both antibodies are directed against apolipoprotein(a) and not Lp(a).

The assay described here offers some advantages over other methods for Lp(a) quantification. In contrast to a similar assay described recently (23), we used the biotin streptavidin system with europium labelled streptavidin for the detection of the second antibody. *Jürgens* et al. (23) labelled their polyclonal anti apolipoprotein B or anti Lp(a) antibodies directly with europium. However, this is expensive because of the high price of the europium labelling reagent. Biotinylation of the antibody and europium labelling of streptavidin reduces the cost, because biotin is cheap and every labelled streptavidin molecule can, at least in theory, react specifically with biotin.

Our method was not affected by triacylglycerols (tab. 2) or haemolysis (tab. 1). The interference of plasminogen was neglegible (s. results). The assay offers a wide measuring range and a lower detection limit, which is satisfactory for both clinical and research purposes. The accuracy and the easy and rapid performance make the method well suited for routine use.

Even if there is still some discussion about the clinical validity of Lp(a), this analyte is frequently measured. No "gold standard" exists for quantifying Lp(a). In this paper we describe a method, which exclusively makes use of commercially available reagents and which includes the biotin steptavidin system. Therefore this assay can be performed in many laboratories, e. g. with use of streptavidin-enzyme or -isoluminol labelling, reagents which are both commercially available.

We compared the TRIFMA (25) with an EIA and with the EID-method and found a good correlation with both methods (figs. 2 and 3). Differences, however, were seen at low and high concentrations of Lp(a). This can be explained by the superior ability of the TRIFMA to detect low and high Lp(a) levels. The Lp(a) assay described here has been successfully used as routine method for quantifying Lp(a) in our laboratory for more than 1½ years.

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